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RESEARCH PAPER

Arbuscular mycorrhizal fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region

K.P. Radhika • B. F. Rodrigues

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Abstract: Thirty-six medicinal plant species belonging to 25 families were surveyed to study Arbuscular mycorrhizal (AM) fungal diversity from different localities of North and South Goa of Western Ghats, Goa region, India. A total of 30 medicinal plant species were found to be mycorrhizal and six plant species showed absence of AM fungal colonization. Forty two AM fungal species belonging to five genera *viz*., *Glomus, Acaulospora, Scutellospora, Gigaspora and Ambispora* were recovered from the rhizosphere soil. *Glomus* was found to be the most dominant genera in the study sites, and *Glomus fasciculatum* the most dominant AM fungal species. Negative significant correlation was observed between percent colonization and spore density. Simpson's and Shannon Weiner diversity index studies carried out in North and South Goa exhibited less variation in both the sites, indicating a stable and a diverse plant community.

Keywords: Arbuscular mycorrhizal fungi; *Glomus;* medicinal plants; Western Ghats; Shannon Weiner diversity index; spore density

Introduction

India is endowed with a rich wealth of medicinal plants. Although a good proportion of the medicinal plant species do occur throughout the country, peninsular Indian forests and the Western Ghats are highly significant with respect to varietal richness (Parrota 2001). Medicinal plants are important for pharmacological research and drug development, not only as plant constituents used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee 2003). It is reported that in India, 4 365 ethnic communities, including over one million folk healers, use around 8 000 species of medicinal plants. They are increasingly becoming economically important due to the growing demand for herbal products in the domestic and global market.

Across the country, the forests are estimated to harbour 90% of the country's medicinal plant diversity, and only about 10% of the known medicinal plants of the country are restricted to non-forest habitats. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-toxic, having no side effects and easy availability at affordable prices. Due to an increasing demand for medicinal plants and to a loss and fragmentation of natural habitats, close to 300 species of Indian medicinal plants have been so far assessed as under threat in the wild (based on International Union for Conservation for Nature (IUCN) Red List Criteria). Around 1,000 species are estimated to be facing various degrees of threat across different biogeographic regions in the country (Seth and Sharma 2004).

Arbuscular mycorrhizal (AM) fungi are major component of rhizosphere microflora in natural ecosystems and play significant role in the re-establishment of nutrient cycling (Peterson et al. 1985). They modify the structure and function of plant communities (Douds and Miller 1999) and are useful indicators of ecosystem change (McGonigle and Miller 1996).

Earlier studies on the occurrence of Arbuscular mycorrhizal fungi in medicinal plants mostly concentrate on rhizomes (Taber and Trappe 1982; Selvaraj et al. 1986). Later, Nasim (1990), Udea et al. (1992), Gautam and Sharma (1996), Rani and Bhaduria (2001), Selvaraj et al. (2001), Muthukumar et al. (2001), and Panwar and Tarafdar (2006) reported the occurrence of medicinal plants from India.

The Western Ghats, a valuable repository for biodiversity after the Himalayas, is one of the 34 mega diversity hot spots of the world. It contains 4 000 (27%) of the country's plant species, of which 38% (1 500 species) are endemic. The high biodiversity of the Western Ghats could be attributed to its varied habitat types ranging from semi-arid grasslands to tropical rainforests. Recently mycorrhizal association in several plant species from different habitat types from Western Ghats region of Southern

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K.P. Radhika • B. F. Rodrigues (✉)
Department of Botany, Goa University, Goa- 403206, India.
Email: radhikanair15@gmail.com

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India were reported by Appasamay and Ganapathi (1995), Muthukumar et al. (1996), Muthukumar and Udaiyan (2001), Khade et al. (2002) and Bukhari et al. (2003). However, the species diversity and composition of Arbuscular mycorrhiza fungal communities from medicinal plants of the Western Ghats of Goa region is largely unknown. Therefore, the present work was undertaken to study the AM fungal diversity in medicinal plant species of Western Ghats, Goa.

Materials and methods

Study sites

Roots and rhizosphere soil of selected medicinal plant species were collected between January 2004 and March 2006 from different localities of both North and South Goa of Western Ghats region (Fig. 1). The climate is tropical, warm and humid with laterite, lateritic, clayey-loamy soil. Mean temperature range from 20°C–35°C, with an average rainfall of 2 500 mm.

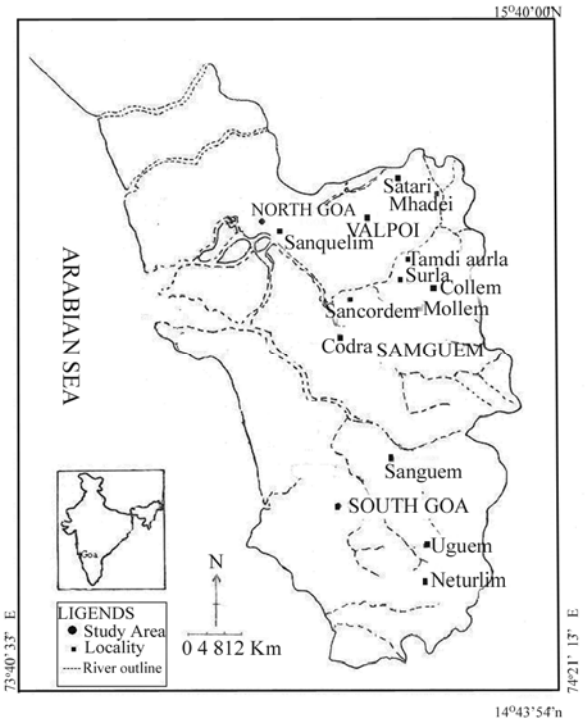

Fig. 1 Map of Goa showing the study sites in Western Ghats.

Sample collection

Thirty-six medicinal plant species along with the rhizosphere soil were collected from different localities belonging to 25 families from both North and South Goa of Western Ghats region. Collections of the samples were carried out in all the three seasons *viz*., monsoon (June-October), post monsoon (November-January) and pre-monsoon (February-May). Both wild (19) and cultivated (17) plant species were selected for the study. Identification of the plant species was carried out based on the flora of Rao (1985) and Mathew (1991).

Soil analysis

For soil analysis, samples were collected from a soil depth of 0–25 cm from different locations of North and South Goa, and were brought to the laboratory in polyethylene bags. Samples were passed through a 2-mm sieve to remove larger soil particles and were mixed thoroughly to obtain a composite sample.

Soil pH was measured in 1:2 soil water suspension using pH meter (LI 120 Elico, India). Electrical Conductivity was measured at room temperature in 1:5 soil suspension using Conductivity meter (CM-180 Elico, India). Standard soil analysis techniques *viz*., Walkley and Black (1934) rapid titration method and Bray and Kurtz method (1945) were employed for determination of organic carbon and available phosphorus, respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel 1952) using flame photometer (Systronic 3292). Available zinc, copper, manganese and iron were quantified by DTPA-$CaCl_2$- TEA method (Lindsay and Norvell 1978) using Atomic Absorption Spectrophotometer (AAS 4139).

Estimation of arbuscular mycorrhiza fungal colonization

Composite rhizosphere soil samples prepared for each of plant species were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed for AM fungal colonization whereas soil samples were stored in deep freezer at 4°C until analyzed. Fixed roots were placed in 2.5% KOH (Koske and Gemma 1989) acidified with 1%HCl and stained with trypan blue. The stained roots were examined with a compound microscope (100X-1,000X) for AM fungal structures and percentage root length colonization was estimated according to slide method (Giovannetti and Mosse 1980). A segment was considered mycorrhizal when it showed the presence of hyphae and arbuscule or vesicle.

Isolation of Arbuscular mycorrhizal fungal spores

Arbuscular mycorrhizal fungal spores were isolated by modified method of wet sieving and decanting technique (Muthukumar et al. 1996). Intact and crushed spores in Poly Vinyl-Lacto Glycerol (PVLG) (Koske and Tessier 1983) were examined under Olympus BX 41 microscope and were identified based on spore morphology and sub cellular characters.

Taxonomic identification of spores

Intact and unparasitized spores were used for the quantification of spore density and taxonomy of Arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi were identified according to their spore morphology and wall characteristics (Schenck and Perez 1990; Morton and Benny 1990; Almeida and Schenck 1990; Bentivenga and Morton 1995; Walker and Vestberg 1998; Redecker et al. 2000; Morton and Redecker 2001). Taxonomic identification of spores was also carried out by matching the

descriptions provided by the international culture collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu.).

Diversity studies

Diversity studies were carried out in North and South Goa separately for abundance and diversity of Arbuscular mycorrhiza fungal species. Species richness is the number of species present in an ecosystem. Simpson's diversity index AM fungal species diversity and abundance was calculated using Simpson's Diversity index *1- D* (Simpson 1951), $D=1-\Sigma (P_i)^2$ where $P_i = ni/N$, (ni) the relative abundance of the species, is calculated as the proportion of individuals of a given species (*ni*) to the total number of individuals in a community (*N*). Simpson's reciprocal index was calculated using the following formulae, 1/D**.** Shannon diversity index (*H*) is commonly used to characterize species diversity in a community, which accounts for both abundance and evenness of the species present, $H= -\Sigma (P_i \ln (P_i)$ (Shannon and Weaver 1949). Species evenness is calculated by the following formulae, $E_{(H)} = H/H_{max}$ where $H_{max} = \ln S$, S= total number of species in the community (richness).

Statistical analysis

Pearson correlation coefficient was performed to assess the relationship between root colonization and spore density. Statistical analysis for correlation coefficient was carried out using WASP (Web Based Agricultural Package) 0.2. For the analysis, difference were considered significant when $P \leq 0.05$.

Results

Results of soil characteristics of the both the sites are depicted in Table 1. The soil pH was found to be acidic and ranged from 5.7 to 5.8. Electrical Conductivity ranged from 0.048 to 0.049 m/mhos. Organic carbon content was higher in the soils of South Goa (1.67%) as compared to the soils analysed from North Goa (0.39%). Soils at both the sites were deficient in available P. Available potassium ranged from 268.8–604.88 kg/ha at both sites while levels of micronutrients like Cu, Zn, Fe and Mn varied at both the sites.

Table 1. Soil sample analysis of North Goa and South Goa

Macronutrients Parameters	pH	Electrical Conductivity m/mhos	Organic carbon %	Phosphorus P_2O_5 Kg/Ha	Potassium K_2O Kg/Ha
North Goa	5.6-5.8	0.049	0.39	Traces	604.8
South Goa	5.5-5.7	0.048	1.67	9.81	268.8
Micronutrients (ppm)	Zinc (Zn)	Iron (Fe)	Manganese (Mn)	Copper (Cu)	Boron (B)
North Goa	0.72	16.24	17.58	0.48	1.3
South Goa	4.2	71.6	83	9.02	0.84

Arbuscular mycorrhizal colonization was recorded in 30 plants out of 36 medicinal plant species undertaken for the study. The Arbuscular mycorrhiza colonization was characterized by arbuscules and/or vesicles and intraradical hyphae. Both arum and paris types of arbuscules were observed. In arum type morphology, hyphae mostly extended intercellularly (longitudinal hyphae) and formed arbuscules, whereas in paris type morphology, compound arbuscles and arbusculate coils were observed. No colonization was recorded in *Commelina benghalensis*, *Physalis minima*, *Adathoda vasica*, *Murraya koenigii*, *Piper nigrum* and *Euphorbia pulcherrima* (Table 2 & 3). Hyphal and vesicular colonization were observed in 16 plant species whereas four plant species exhibited arbuscular colonization. Hyphal, vesicular and arbuscular colonization were recorded in 10 plant species. Maximum percent colonization was found in *Azadirachta indica* and *Cajanus* sp. (100%) and the minimum was recorded in *Alpinia galanga* (8.33%) (Table 2 & 3).

Table 2. List of medicinal plants surveyed for Arbuscular mycorrhizal fungal association from North Goa.

Sr. No.	Plant species & Family	Locality	Status	AM fungal colonization	colonization (%)
1	*Adathoda vasica* Nees. (Acanthaceae)	Mhadei	Wild	–	–
2	*Andrographis paniculata* Nees.(Acanthaceae)	Valpoi	Cultivated	H, V	40
3	*Azadirachta indica* A Juss. (Meliaceae)	Valpoi	Cultivated	H,V	100
4	*Catharanthus roseus* L. (Apocynaceae)	Valpoi	Cultivated	H, V	70
5	*Centella asiatica* L.(Apiaceae)	Ustae	Wild	H, V, A	90
6	*Commelina benghalensis* L.(Commelinaceae)	Valpoi	Wild	–	–
7	*Curcuma* sp. (Zingiberaceae)	Bhuipal	Wild	H,V	40
8	*Eclipta alba* Hassk. (Asteraceae)	Valpoi	Wild	H,V,A	95
9	*Garcinia indica* Choisy. (Clusiaceae)	Valpoi	Cultivated	H,V	95
10	*Hibiscus rosa-sinensis* L. (Malvaceae)	Valpoi	Cultivated	H, V	20
11	*Impatiens balsamina* L. (Balsaminaceae)	Sanquelim	Wild	H, V, A	85
12	*Lawsonia inermis* L. (Lytharaceae)	Mhadei	Cultivated	H, V	20
13	*Leucas aspera* L. (Apocynaceae)	Ustae	Wild	H, V	87
14	*Physalis minima* L. (Solanaceae)	Valpoi	Cultivated	–	–

Legend: H= hyphal colonization, V = vesicular colonization, A= arbuscular colonization, – = no colonization.

Forty-two Arbuscular mycorrhiza fungal species belonging to five genera *viz*., *Glomus* (24), *Acaulospora* (8), *Scutellospora* (7), *Gigaspora* (2), and *Ambispora* (1) were recovered from the rhizosphere soil samples with the species number given in

parenthesis. Spore density varied from 1 197 spores (*Hemidesmus indicus*) to 14 spores (*Eclipta alba*) per 100 g soil (Table 4 & 5). The study reveals that the wild medicinal plant species showed higher spore diversity as compared to the cultivated medicinal plants. The most dominant genera recorded in the present study were *Glomus* followed by *Acaulospora, Scutellospora, Gigaspora* and *Ambispora*. *Glomus fasciculatum* was found to be the most dominant Arbuscular mycorrhiza fungal species followed by *A. scrobiculata*.

Table 3. List of medicinal plants surveyed for Arbuscular mycorrhizal fungal association from South Goa.

Sr. No.	Plant species & Family	Locality	Status	AM fungal colonization	Colonization (%)
1	*Aloe vera* L. (Liliaceae)	Uguem	Cultivated	H, V, A	8.33
2	*Alpinia galanga* (L.) Sw. (Zingiberaceae)	Mollem	Wild	H, V	21
	Artemisia vulgaris L. (Asteraceae)	Sancordem	Cultivated	H, A	66.67
4	*Asparagus officinalis* L. (Leguminosae)	Surla	Wild	H,V,A	40
5	*Bryophyllum pinnatum* (Lam.) Kurz.(Crassulaceae)	Sadolxem	Cultivated	H,V	65.22
6	*Cajanus* sp. (Leguminosae)	Surla	Wild	H, A	100
7	*Clitoria ternatea* L. (Leguminosae)	Sadolxem	Cultivated	H, V, A	77.7
8	*Curculigo orchidoides* Gaertn. (Amaryllidaceae)	Mollem	Wild	H, V	22.8
9	*Curcuma decipiens* Dalz. (Zingiberaceae)	Sancordem	Wild	H, V	29.34
10	*Cymbopogon citrates* Stapf. (Poaceae)	Sadolxem	Cultivated	H, V	20
11	*Euphorbia hirta* L. (Euphorbiaceae)	Codra	Wild	H, V, A	_
12	*Euphorbia pulcherrima* Willd. (Euphorbiaceae)	Codra	Wild	—	_
13	*Hemidesmus indicus* R. Br. (Asclepiadaceae)	Sanguem	Wild	H, V, A	34
14	*Ixora coccinea* L. (Rubiaceae)	Tamdi Surla	Cultivated	H, V	24
15	*Mentha* sp. (Lamiaceae)	Sadolxem	Cultivated	H, V	30
16	*Mimosa pudica* L. (Leguminosae)	Codra	Wild	H, V, A	98
17	*Murraya koenigii* (L.) Spr. (Rutaceae)	Sadolxem	Cultivated	_	_
18	*Naregamia alata* W. & A. (Meliaceae)	Uguem	Wild	H, A	60
19	*Ocimum sanctum* L. (Lamiaceae)	Tamdisurla	Cultivated	H, V, A	66
20	*Phyllanthus niruri* L. (Euphorbiaceae)	Tamdi Surla	Wild	H, A	50
21	*Piper nigrum* L. (Piperaceae)	Neturlim	Cultivated	—	79
22	*Rauwolfia serpentina* (L.) Benth. (Apocynaceae)	Tamdi Surla	Wild	H, V	40

Legend: H= hyphal colonization, V = vesicular colonization, A= arbuscular colonization, — = no colonization.

Table 4. Spore density and Arbuscular mycorrhizal fungal species identified from rhizosphere soil samples of selected medicinal plants from North Goa.

Plant species	Spore density*	Arbuscular mycorrhizal fungal spores
Andrographis paniculata	93	*A. scrobiculata, G. aggregatum*
Commelina benghalensis	30	*A. myriocarpa, G.* sp.
Physalis minima	33	*A. rehmii*, *G. fasciculatum*, *G. multicaule, G. maculosum, G. geosporum, G. rubiforme*
Catharanthus roseus	52	*A. laevis, G. dimorphicum*
Leucas aspera	20	*G. rubiforme*, *G. formosanum*, *G. fasciculatum*
Centella asiatica	80	*G. multicaule*, *G. clarum*, *G. fasciculatum*, *A.delicata*, *S. scutata*
Impatiens balsamina	45	*G.microcarpum*, *G. fasciculatum*
Hibiscus rosa- sinensis	62	*G. maculosum*, *G. glomerulatum, A. scrobiculata*
Garcinia indica	27	*A. scrobiculata, G. fasciculatum, Gi.* sp.
Azadirachta indica	72	*A. scrobiculata, G. fasciculatum, Gi. albida, S. calospora*
Adathoda vasica	35	*A. scrobiculata, G. geosporum, , G. multicaule, G. fasciculatum*
Eclipta alba	14	*A. scrobiculata, Am. leptoticha, G. fasciculatum, S. heterogama*
Curcuma sp.	200	*Am. leptoticha, G. rubiforme, G. geosporum, G. fasciculatum, G. aggregatum*
Lawsonia inermis	61	*A. scrobiculata, G .multicaule, G. intraradices*

Legend: *A= Acaulospora*, *Am= Ambispora, G= Glomus, Gi= Gigaspora, S= Scutellospora* *Spores $100g^{-1}$ of soil.

Medicinal plant species exhibited higher root colonization levels during pre monsoon and least during monsoon. The percent root colonization varied throughout the season with highest (83%) during April and least during June (38.9%). Arbuscules were relatively infrequent and were observed in few plant species in all the seasons, whereas vesicular colonization was observed in all the plant species (Fig. 2).

The spore density of Arbuscular mycorrhiza fungi varied in different seasons. High mean spore density was observed during the monsoon period (August) and the least during the post-

monsoon period (January) (Fig. 3). Weak negative non-significant correlation was found between percent colonization and spore density (r= -0.1, p≤0.05).

Table 5. Spore density and Arbuscular mycorrhizal fungal species identified from rhizosphere soil samples of selected medicinal plants from South Goa.

Plant species	Spore density*	Arbuscular mycorrhizal fungal spores
Aloe vera	421	*G. maculosum, G. multicaule*, *G. geosporum*
Alpinia galanga	76	*G. caledonium, G. mosseae, G. fasciculatum, G. geosporum, Am. leptoticha*
Artemisia vulgaris	183	*G. fasciculatum, G. magnicaule, G. geosporum*
Asparagus officinalis	149	*A. scrobiculata, G. rubiforme, G. fasciculatum, G. geosporum*
Bryophyllum pinnatum	166	*A. laevis, G. multicaule, G. aggregatum, Gi. albida*
Cajanus sp.	231	*A. nicolsonii, A. scrobiculata, G. constrictum, G. multicaule, G. fasciculatum*
Clitoria ternatea	94	*A. spinosa, G. multicaule, G. glomerulatum, G. fasciculatum, Gi. albida*
Curculigo orchidoides	256	*G. fasciculatum, G. intraradices*
Curcuma decipiens	238	*Am. leptoticha, G. constrictum, G. fasciculatum, G. caledonium G. geosporum, G. multicaule*
Cymbopogon citrates	37	*G. fasciculatum, G. aggregatum, G. multicaule*
Euphorbia hirta	120	*A. scrobiculata, G. geosporum, Gi. albida*
Euphorbia pulcherrima	125	*A. scrobiculata, G. geosporum, G. albidum, G. mosseae*
Hemidesmus indicus	1197	*Am. leptoticha, G. maculosum, G. geosporum, G. multicaule, G. fasciculatum*
Ixora coccinea	40	*A. nicolsonii*, *A. scrobiculata, G. etunicatum*, *G. aggregatum, S. biornata*
Mentha sp.	85	*A. scrobiculata, G. citricola, G. fasciculatum, G. multicaule*
Mimosa pudica	50	*Am. leptoticha, G. aggregatum*, *G. glomerulatum*, *G. geosporum*, *G. intraradices*
Murraya koenigii	91	*G. fasciculatum, G. multicaule, G. halon*
Naregamia alata	614	*A. scrobiculata, Am. leptoticha, A. nicolsonii, G. rubiforme, G. maculosum, G. fasciculatum*, *S. verrucosa*
Ocimum sanctum	50	*G. fasciculatum*, *G. macrocarpum*
Phyllanthus niruri	40	*G. arborense, A. tuberculata, S. gregaria*
Piper nigrum	415	*Am. leptoticha, A. scrobiculata*, *G. multicaule*, *G. intraradices*, *G. geosporum*, *G. flavisporum, G. fasciculatum*, *S. pellucida*
Rauwolfia serpentina	20	*G. maculosum, G. fasciculatum*

Legend: *A= Acaulospora*, *Am= Ambispora, G= Glomus, Gi= Gigaspora, S= Scutellospora* *Spores 100g^{-1} of soil.

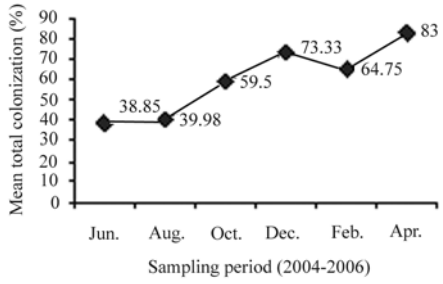

Fig. 2 Seasonal variation in percent colonization of Arbuscular mycorrhiza fungal species in medicinal plants.

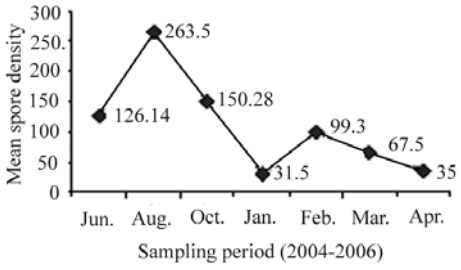

Fig. 3 Seasonal variation in Arbuscular mycorrhiza fungal spore density in medicinal plants.

In South Goa, 27 AM fungal species belonging to five genera *viz*., *Glomus, Acaulospora, Scutellospora*, *Gigaspora* and *Ambispora* were recovered from the rhizosphere soil. Maximum spore density was recorded in *Curcuma* sp. (200 spores) and the minimum in *Eclipta alba* (14 spores per 100 g soil) (Table 3). In North Goa, 26 AM fungal species belonging to five genera *viz*., *Glomus, Acaulospora, Scutellospora*, *Gigaspora* and *Ambispora* were recorded. Maximum spore density was observed in *Hemidesmus indicus* (1,197 spores) and the least in *Rauwolfia serpentina* (20 spores per 100 g soil) (Table 4). Species richness of Arbuscular mycorrhiza fungi was found more in South Goa (eight species/site) compared to North Goa (six species/site). Diversity index studies *viz*., Simpsons Diversity Index and Shannon Wiener Diversity Index showed that Arbuscular mycorrhiza fungal species dominance in South Goa (0.95, 2.88) was slightly higher than that in North Goa (0.9, 2.86) (Fig. 4A & B). Complete evenness of Arbuscular mycorrhiza fungal species was found in North Goa (1) compared to South Goa (0.93) (Fig. 4C).

Discussion

In the present study, rhizosphere soil at both sites showed less amount of available P and it could be due to the fact that tropical soils are P fixing. It is reported that nearly 80–85% of P applied

to the soil is made unavailable to plants because of its inaccessibility due to fixation and immobilization (Rosalind Padma and Kandaswamy 1990). If phosphorus is scarce, mycorrhizas are abundant but if such elements are readily available colonization is reduced. Further organic carbon content and available potassium content of the rhizosphere soil was high and not influenced by management regimes. Earlier workers have reported that mycorrhizal response is greater in soil with lower amount of Zn, Cu, Fe and Mn (Sreenivasa and Bagyaraj 1988). Micronutrients were present in lower concentrations except manganese and iron. A possible reason could be that quantity wise, iron is the most abundant micronutrient followed manganese, zinc, copper, boron and molybdenum and their availability is more in acidic soils as compared to neutral and alkaline soils (Tandon 1994).

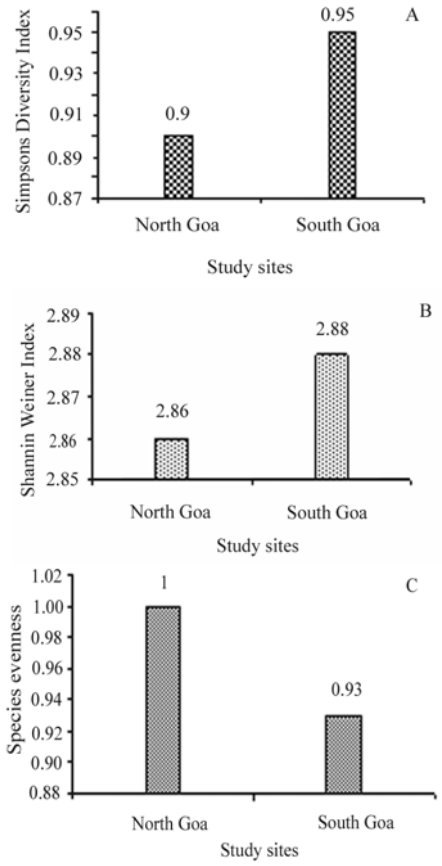

Fig. 4 Diversity indices of Arbuscular mycorrhiza fungi in medicinal plants at the study sites A) Simpson's diversity Index B) Shannon Wiener Index C) Species evenness

The present study confirms the presence of Arbuscular mycorrhiza colonization in medicinal plants. Similar observations were recorded earlier (Taber and Trappe 1982; Srivastava and Basu 1995; Muthukumar and Udaiyan 2001; Gorsi 2002). This study contradicts the earlier findings of Muthukumar and Udaiyan (2001) who reported that the proportion of non-mycorrhizal species in the Western Ghats is high compared with other vegetation world wide, whereas in the present study 83.33% of the plant species were mycorrhizal. Two medicinal plants lacking Arbuscular mycorrhiza colonization belonged to non-mycorrhizal families *viz*., Commelinaceae and Euphorbiaceae (Tester et al. 1987; Brundrett 1991) where as other plant species lacking mycorrhizas belonged to families reported mycorrhizal *viz*., Solanaceae, Acanthaceae, Rutaceae and Piperaceae. This may be due to the presence of fungitoxic compounds in root cortical tissue or in root exudates that may reduce susceptibility of plants to mycorrhization (Tester et al. 1987).

The mycorrhizal colonization differed among medicinal plant species and there was a considerable variation in percent root colonization and number of different Arbuscular mycorrhiza fungal spores associated with rhizosphere soil but no definite correlation could be established between them, which is in agreement with the findings of Kalita et al. (2002). This could be due to the fact that Arbuscular mycorrhiza fungal sporulation is dependent on a wide range of host fungal and environmental factors, and their germination potential varies at different times of the year (Tommerup 1983; Koske and Gemma 1988). The presence of mycorrhizal colonization in all the seasons indicates that the plant species are dependent on mycorrhizae during the entire year. The studies that have reported seasonality of the mycorrhizal association generally assume a direct influence of environmental conditions such as temperature and moisture, phenology and physiological status of the plant (Mohammad et al. 1998; Brundrett 2002).

In the present study, Arbuscular mycorrhizal (AM) fungi displayed little or no host specificity. However, earlier studies clearly demonstrated that the community composition of Arbuscular mycorrhiza fungal was affected by environmental factors and vegetation (Brundrett 1991). This indicates that Arbuscular mycorrhizae may prefer certain habitats. The possible reasons for the predominance of *Glomus* sp. are that spores of *Glomus* species have different temperature and pH preferences for germination (Wang et al. 1997) and *Acaulospora* species are often associated with acidic soils (Morton 1986; Abbott and Robson 1991). Secondly, *Gigaspora* species predominate in soils with a high sand content, especially dunes (Day et al. 1987; Lee and Koske 1994); the genus *Scutellospora* is ancestral to *Gigaspora* (Walker 1992) and probably prefers similar sandy soils. Dominance of genus *Glomus* from medicinal plants has been reported earlier Selvaraj et al. (2001).

Forty two Arbuscular mycorrhiza fungal species belonging to five genera were recovered from the rhizosphere soil in the present study. This is in agreement with the findings of Francis and Read (1994) and Allen et al. (1995) who reported high Arbuscular mycorrhiza fungal species diversity in medicinal plants. However Muthukumar et al. (2001) reported only 35 Arbuscular mycorrhiza fungal species from 329 medicinal plant species from Western Ghats.

Large variation in spore number (14–1,197 spores 100 g^{-1} soil) recorded in the rhizosphere soil can be attributed to several reasons. Firstly, the occurrence of several Arbuscular mycorrhiza fungi in the soils or within roots suggest that interspecific competition between them is possible (Brundrett and Kendrick

1990). Secondly, subsequent variation in the spore production occurs among Arbuscular mycorrhiza fungi associated with host plants, suggesting that competition between fungi and environmental factors influence spore production in natural communities (Gemma and Koske 1988). Also peak period of spore production is generally thought to coincide with the period of fungal resource remobilization from senescing roots (Sutton and Barron 1972) and is greatest in natural communities when roots activity is interrupted by a long dry season (Janos 1980).

Arbuscular Mycorrhizal spore numbers were strongly seasonal with increasing numbers of Arbuscular mycorrhiza fungal spores as the rainy season progressed. Similar seasonal patterns in spore number were observed in earlier studies resulting in gradual increase in the spore numbers during the rainfall period, followed by a decrease during the dry period (Martin et al. 1999; Miranda et al. 1997). Arbuscular mycorrhizal spore density was found to be higher in wild medicinal plant species as compared to cultivated species. This observation could be attributed to the undisturbed nature of the ecosystem.

Species richness was the maximum in *Piper nigrum* where eight Arbuscular mycorrhiza fungal species belonging to three genera were recovered from the rhizosphere soil at both the sites. This may be due to the fact that species richness index is reported to be dependent on the sample size, as more number of samples are collected, spores of more species are likely to be recovered (Sturmer and Bellei 1994). Diversity studies showed less variation in both sites indicating a stable and a diverse plant community (Fower and Antonovics 1981). Increase in species richness was accompanied by an increase in plant diversity and ecosystem productivity. Arbuscular mycorrhizal (AM) taxa have a specific multidimensional niche determined by the plant species that are present at the site and by edaphic factors such as pH, moisture content, and phosphorus (P) and nitrogen (N) availability (Ahulu et al. 2006). As a result there is a large variation in the composition of Arbuscular mycorrhiza fungal taxa between and within the site (Burrows and Pfleger 2002; Hart and Klironomos 2002). Climatic seasons seem to be more influential on distribution and abundance of mycorrhizal spores. Mycorrhization of forest plants have recently been considered as the substitute of chemical fertilization regarding environment pollution and disease control for better management of tropical forests (Dhar and Mridha 2006).

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